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INVITED REVIEW

Aberrant crypt foci in colorectal carcinogenesis. Cell and crypt dynamics

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Abstract. Aberrant crypt foci (ACF) have been identified on the colonic mucosal surface of rodents treated with colon carcinogens and of humans after methylene-blue staining and observation under a light microscope. Several lines of evidence strongly suggest that ACF with certain morphological, histological, cell kinetics, and genetic features are precursor lesions of colon cancer both in rodents and in humans. Thus, ACF represent the earliest step in colorectal carcinogenesis. This paper has the main purpose of reviewing the evidence supporting this view, with particular emphasis on cell and crypt dynamics in ACF. ACF have been used as intermediate biomarkers of cancer development in animal studies aimed at the identification of colon carcinogens and chemopreventive agents. Recently, evidence has also shown that ACF can be effectively employed in chemopreventive studies also in humans.

INTRODUCTION

Colorectal carcinogenesis is a stepwise process, which leads from normal mucosa to the development of carcinoma, through a series of genetic alterations (Vogelstein *et al*. 1988), although some of these may be effects rather than causes of the neoplastic transformation (Prehn 1994). The process of carcinogenesis is usually very long, taking many years, if not decades, to reach the invasive stage, and is the result of the effects of environmental or genetic injuries and of the adaptive responses of the host (Farber & Rubin 1991). Thus, there should be time to take adequate measures to prevent the onset of cancer. Unfortunately, however, at the present time colorectal cancer remains a major cause of death in most developed countries.

One of the earliest events in colorectal carcinogenesis is the alteration of the proliferative pattern of epithelial cells in the colonic crypts (Lipkin 1988). In addition, evidence has been provided on the impairment of the control of programmed cell death in the colonic epithelium of patients at risk of colon cancer (Bedi *et al*. 1995), suggesting that malfunction of the mechanisms controlling cell kinetics underlies the first steps of colorectal

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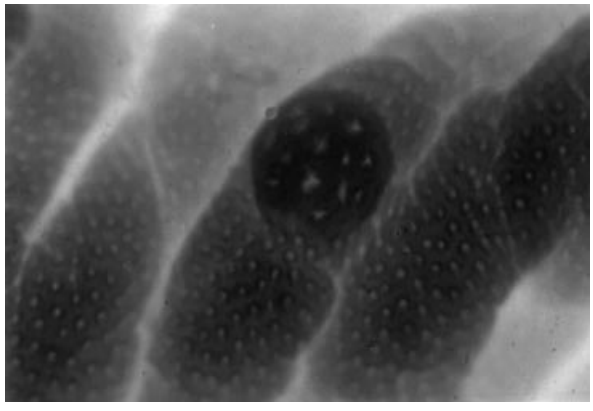

Figure 1. An aberrant crypt focus is evident in the centre of the figure. Crypts are larger and darker than normal after staining with methylene-blue and observation under a light microscope at 40 ×. Luminal openings of aberrant crypts are larger than normal, and the shape is irregular.

carcinogenesis. The result is the growth of polyps, which can be observed and removed during colonoscopic examination of the mucosal surface of the large bowel. It is widely accepted that dysplastic polyps, i.e. adenomas, are precursors of colorectal cancer (Muto, Bussey & Morson 1975).

However, the earlier morphological steps of colorectal tumour formation are not yet known. Most information on this topic comes from experimental carcinogenesis and from human pathological studies. Histological changes in normal-appearing colonic mucosa far from the tumour were previously observed both in animals treated with colon carcinogens (Deschner 1974), and in humans with colon cancer (Shamsuddin *et al*. 1981). However, in the mid-1980s, Ranjana Bird examining the methylene-blue-stained mucosal surface of azoxymethane-treated mice under the light microscope, for the first time described crypts which appeared larger, thicker, and darker than normal (Bird 1987). They were referred to as aberrant crypts, and considered putative precursors of experimental colon cancer. They tend to cluster in aggregates, called aberrant crypt foci (ACF) (Tudek, Bird & Bruce 1989), which increase in size with time. Indeed, aberrant crypts undergo a replication process, as normal crypts (Chang 1984), which starts at the bottom with budding and then branching until the generation of two new crypts (McLellan, Medline & Bird 1991a, Fujimitsu *et al*. 1996).

ACF have been described also in the human colon (Roncucci *et al*. 1991a, Pretlow *et al*. 1991)(Figure 1). They have features resembling those of rodents. Aberrant crypts are larger and darker than normal after methylene-blue staining, and they cluster in foci which are slightly bulging on the normal mucosal surface. Also, human ACF increase in size by a mechanism of crypt fission as in normal mucosa (Cheng *et al*. 1986). Thus, it seems evident that colonic epithelial cell and crypt kinetics are crucial for the development of focal lesions in the colon.

This paper reviews the evidence supporting the contention that ACF are precursors of colon cancer in rodents and in humans with particular emphasis on cell and crypt dynamics. Firstly, cell proliferation and apoptosis will be described briefly in normal colonic mucosa along with the known mechanisms of genetic control, and then the morphological, histological, biochemical, cell kinetics and genetic alterations in experimental and human aberrant crypt foci will be discussed.

CELL KINETICS OF NORMAL COLORECTAL MUCOSA

Intestinal crypts are invaginations of the surface epithelium, creating structures which resemble test tubes. In sections taken perpendicular to the surface they are lined up in parallel with the mouth open to the intestinal lumen (Levine & Haggitt 1989).

Studies in animals and humans have shown that cells proliferate at the bottom of colonic crypts (Lipkin & Quastler 1962, Lipkin, Sherlock & Bell 1963), they differentiate while migrating up along the crypt axis, losing their ability to divide, and once they reach the surface, they die and are extruded into the intestinal lumen. In normal conditions the proliferative zone of crypts is limited to the lower two thirds with the major zone of proliferation located in the lower third, along the longitudinal axis of the crypt (Cole & McKalen 1961, Deschner 1980, Potten *et al*. 1992a). The cell population of normal crypts arises from single stem cells located at the very bottom of colonic crypts (Potten 1992), as confirmed by the demonstration of monoclonality of colonic crypts both in mice (Ponder *et al*. 1985, Griffiths *et al*. 1988) and in humans (Endo, Sugimura & Kino 1995).

The normal proliferative status of the colorectal mucosa has a circadian cicle (Kennedy *et al*. 1985, Buchi *et al*. 1991) and is modulated by several factors, including age (Roncucci *et al*. 1988), dietary factors (Stadler *et al*. 1988), hormones (Tutton & Barkla 1987, 1988), drugs such as laxatives (Lehy, Abitbol & Mignon 1984, Kleibeuker *et al*. 1995), and other factors not thoroughly known.

Altered proliferative activity in colonic epithelial cells has been described in animals treated with colon carcinogens (Thurnherr *et al*. 1973), along with cytological and histological changes (Chang 1984). Initially, there is an expansion of the proliferative zone to the whole crypt, including the upper, superficial compartments, and even the surface epithelium of the colon; in this phase the main zone of DNA synthesis is still limited to the lower third. Then, in a further development, the major zone of proliferation shifts to the upper portions of the crypt. This is in line with an overall increase in the fraction of replicative cells over total epithelial cells of the crypt (labelling index, as measured with [^{3}H]thymidine incorporation into DNA, or other compounds which identify S phase or cycling cells).

Cell proliferation of flat colorectal mucosa is altered also in patients with colonic tumours and in patients at a genetic or environmental risk of cancer of the large bowel (Deschner, Lewis & Lipkin 1963, Deschner & Raicht 1981, Lipkin *et al*. 1983, Biasco *et al*. 1984, Terpstra *et al*. 1987, Ponz de Leon *et al*. 1988, Scalmati *et al*. 1990, Anti *et al*. 1993, Risio *et al*. 1995, Cats *et al*. 1996). Indeed, patients with adenoma or cancer have an increased total labelling index. Furthermore, the longitudinal distribution of replicative cells along the crypt is also altered. In fact, the proliferative zone of the crypt is expanded toward the lumen, thus encompassing the whole crypt (Maskens & Deschner 1977) and, in some cases, it is even limited only to the upper portions of the crypt.

Apoptosis, a form of programmed cell death, was firstly defined on morphological grounds, using transmission electron microscopy (Kerr, Wyllie & Currie 1972), which remains the gold standard method for the identification of the phenomenon (Payne *et al*. 1995). Cell death in colonic crypts is still largely obscure. It is known that a low level of apoptosis normally occurs in cells located at the bottom of crypts (Potten 1992) and at the luminal surface (Sträter *et al*. 1995). The background level of apoptosis can be modulated by physical (Potten 1977, 1992) and chemical (Piazza *et al*. 1995, Barnes *et al*. 1998) agents, and even by dietary factors (Risio *et al*. 1996, Caderni *et al*. 1998, Premoselli *et al*. 1998). This process is important to remove cells carrying mutations in genes involved in colon carcinogenesis. However, at variance with the small intestine, it seems that apoptosis does not occur in the

stem cell regions of colonic crypts (Potten *et al.* 1992b), suggesting that this protective mechanism does not work in the colon. This fact can contribute to explanation of why small intestinal tumours are so rare in humans. Moreover, in colonic adenomas and carcinomas the level of apoptosis is reduced, and can not balance the increased proliferation that occurs in tumours. Indeed, apoptosis is also involved also in the growth of adenomas, thus contributing to the development of cancer (Arai & Kino 1995). It has been reported that goblet cells of normal colorectal mucosa of patients with colon cancer have reduced bile acid-induced apoptosis as compared with non-cancer patients (Garewal *et al.* 1996). This observation suggests that resistance to apoptosis may have a role in colon carcinogenesis.

Cell proliferation and apoptosis are regulated by several genes that have been intensively studied in recent years. It is not the purpose of this review to analyse in detail these genes, but some time should be spent on the adenomatous polyposis coli (*APC*) tumour suppressor gene, whose constitutional mutations cause familial adenomatous polyposis (FAP) (Groden *et al.* 1991, Nishisho *et al.* 1991), a genetic disease inherited as a dominant trait. In this disease, the colon is carpeted by hundreds of adenomas which, if not removed prophylactically, give rise to colon cancer. *APC* should be considered a key gene, because it controls colonic cell kinetics, and thus seems to be involved in the first steps of colon carcinogenesis (Powell *et al.* 1992), in particular in the transition from normal to hyperproliferative mucosa (Kinzler & Vogelstein 1996). Recent data are in line with the hypothesis of a role of the *APC* gene in regulating apoptosis (Morin, Vogelstein & Kinzler 1996, Polyak *et al.* 1996). The gene is very long and complex, and is located on the long arm of chromosome 5 (Bodmer *et al.* 1987, Kinzler *et al.* 1991). *APC* somatic mutations have been shown also in sporadic (non-familial) colonic tumours, probably as early genetic alterations in the development of cancer. Most *APC* mutations (mainly clustered in the long exon 15) produce a truncated malfunctioning protein. The functions of the normal APC protein are only partially known. It is expressed mostly in the upper, more superficial portions of normal colonic crypts where cell proliferation is low or absent (Smith *et al.* 1993). This fact suggests that the protein slows down or blocks cell proliferation. Indeed, the APC protein binds catenins and other proteins (Rubinfeld *et al.* 1993, Su, Vogelstein & Kinzler 1993), and in particular β-catenin. β-catenin is a component of cell–cell adherent junctions (Gumbiner 1996), which, if not down-regulated by the APC protein in epithelial cells (Senda *et al.* 1996), accumulates, activating a positive signal to proliferation, essential for polyp growth. Mutations of the β-catenin gene itself can probably produce the same effect on cell proliferation (Morin *et al.* 1997, Sparks *et al.* 1998).

On the other hand, it has been recently reported that the APC protein also binds to microtubules (Munemitsu *et al.* 1994, Nathke *et al.* 1996), offering new insights into other roles for APC, including apoptosis and cell migration. Indeed, apoptotic cells in culture lose the normal 300 kDa APC protein acquiring a particular shorter 90 kDa protein (Browne *et al.* 1994). Interestingly, a recent report suggests that also the c-*myc* oncogene, which controls cell proliferation, may be regulated by APC (He *et al.* 1998). Indeed, c-*myc* can trigger an apoptotic signal at least in T cell hybridomas (Shi *et al.* 1992).

It is intriguing that other genes that have functions related to the control of cell proliferation have been shown to be involved in the control of apoptosis too, supporting the view that these two phenomena are two sides of the same coin. The prototype of these genes is the p53 tumour-suppressor gene which is frequently inactivated in colorectal carcinomas (Fearon & Vogelstein 1990), although less frequently in earlier lesions. The control of apoptosis seems in part to be mediated through the $p21^{WAF1/CIP1}$ protein, a cyclin-dependent kinase inhibitor (El-Deiry *et al.* 1993).

Other genes playing a role in the control of programmed cell death in the colon are *bcl*-2 and related genes. *bcl*-2 is a negative regulator (Hockenbery *et al*. 1990), whereas *bax* and *bcl*-xS are inducers of apoptosis. In the normal colorectal mucosa, bcl-2 is expressed in the basal epithelial cells of the crypts (Sinicrope *et al*. 1995). Adenomas and carcinomas show a high percentage of bcl-2-positive cells negatively correlated with the apoptotic index, indicating an enhanced inhibition of apoptosis in colorectal tumorigenesis.

EXPERIMENTAL ABERRANT CRYPT FOCI

Since Bird's observation, several animal experiments have been undertaken in order to find evidence of the preneoplastic nature of aberrant crypts. First, ACF were induced by carcinogen treatment only, in a dose and time-dependent manner (McLellan & Bird 1988a,b). Several agents known to be colon carcinogens were able to induce ACF in experimental animals (Bird, McLellan & Bruce 1989), including carcinogens associated with foods (Tudek *et al*. 1989). ACF induction in rodents is also influenced by genetic factors (Moen *et al*. 1996, Feng *et al*. 1997, Papanikolaou *et al*. 1998). Furthermore, agents known to inhibit dimethylhydrazine (DMH) or azoxymethane (AOM)-induced colon cancer can also inhibit ACF induction (McLellan & Bird 1991). The serial histological evaluation of experimental ACF showed different grades of alterations, ranging from mild cellular atypia to overt dysplasia (McLellan *et al*. 1991a). It is not known whether these grades are progressive steps of the same process. ACF with clear dysplasia is probably the immediate precursor of adenoma. Those with mild alterations can progress toward ACF with more severe histological changes, although they may also regress (Shpitz *et al*. 1996).

From a biochemical point of view, ACF show a wide range of enzymatic alterations. They have decreased diaminopeptidase IV, succinate dehydrogenase, and β-galactosidase activities, as measured spectrophotometrically (Sandforth *et al*. 1988). Moreover rodent ACF shows reduced hexosaminidase, *N*-acetyl-β-d-glucosaminidase, and α-naphthyl butyrate esterase activities (Barrow *et al*. 1990, Pretlow *et al*. 1990) and increased γ-glutamyl transpeptidase in the stroma (Barrow *et al*. 1990). The pattern of mucin expression is also altered in ACF. Caderni *et al*. (1995) using the high iron diamine–Alcian blue technique found that sialomucins increase in larger ACF and demonstrated that large ACF are correlated to tumour incidence in a rat model.

The proliferative status of aberrant crypts as determined by autoradiography was shown to be heterogeneous by Bird *et al*. (1989), although no quantitative data were reported. In a further study, aberrant crypts had more cells and a higher total labelling index (LI) than normal surrounding crypts. However, the distribution of S phase cells was similar to normal crypts (McLellan, Medline & Bird 1991b). Later, an increase in the mean proliferative activity of aberrant crypts as compared with normal was shown in F344 rats treated with AOM and injected with 5-bromo-2-deoxyuridine (BrdUrd) before killing (Pretlow, Cheyer & O'Riordan 1994a). However, in this study, the distribution of labelled cells along the longitudinal axis of crypts could not be evaluated. Cell proliferative activity in small ACF (consisting of 2 or 4 crypts) from F344 rats was higher than that of normal crypts both in animals treated with AOM and in controls (Yamashita *et al*. 1994). In this study, the proliferative status was measured with the mitotic index, the proliferating cell nuclear antigen-labelling index (PCNA-LI), and the BrdUrd-labelling index (BrdUrd-LI). Further, PCNA-labelled cells were observed in the upper compartments of aberrant crypts, suggesting that an altered pattern of cell proliferation occurred. In a further study, in Sprague–Dawley rats fed different diets, aberrant crypts were found to be higher than normal, to have higher BrdUrd-LI and mitotic

index, and a shifted proliferative zone toward the lumen (Corpet, Tachè & Peiffer 1997). Interestingly, the transforming growth factor beta-1 (TGFβ1), a cytokine which inhibits epithelial cell proliferation in rodents and humans, was found to be effective in reducing induction and growth of ACF in DMH-treated rats (Mikhailowski *et al.* 1998).

Apoptosis is far less defined in experimental ACF. The number of apoptotic bodies was lower in aberrant than in normal crypts of rats treated with AOM (Magnuson, Shirtliff & Bird 1994). Cholic acid feeding induced resistance to apoptosis both in normal and aberrant crypts. On the other hand, Corpet *et al.* (1997) reported no difference in the number of cells in apoptosis, identified after staining of apoptotic bodies with the Feulgen-fast green method, between normal and aberrant crypts in rats, and diets did not seem to affect the number of apoptotic cells both in aberrant and normal crypts.

ACF grow by a mechanism of crypt fission. Indeed, the number of branching crypts is higher than in normal mucosa. The process can easily be seen also with scanning electron microscopy at the surface of the mucosa (Paulsen *et al.* 1994). The number and size of ACF (number of crypts per focus, also called 'crypt multiplicity') can be modulated by dietary manipulation, enabling the identification of initiators and promotors of experimental colon cancer (Bruce *et al.* 1993). So far the ACF assay has become a useful tool to detect colon carcinogens in the diet (Corpet *et al.* 1990, Caderni *et al.* 1991, Zhang *et al.* 1992, Yang *et al.* 1998), or other risk factors (Koohestani *et al.* 1997), and to identify possible chemopreventive agents (Stamp *et al.* 1993, Takahashi *et al.* 1993, Jenab & Thompson 1998, Rao, Newmark & Reddy 1998). However, no correlation between the number of ACF and the incidence of carcinomas in rats was found (Hardman *et al.* 1991, Magnuson, Carr & Bird 1993), or it was found only in the left colon (Park, Goodlad & Wright 1997). Furthermore there was no relationship between the distribution of ACF and tumours along the large bowel of mice. On the other hand, crypt multiplicity in ACF, i.e. the number of crypts per focus, was a predictor of tumour incidence (Magnuson *et al.* 1993, Caderni *et al.* 1995, Davies & Rumsby 1998). The explanation of this apparent discrepancy is that most ACF regress, and only larger foci progress toward cancer.

Several genetic alterations have been reported in experimental ACF. Most of them are crucial in the control of cell proliferation and cell death. Using *in situ* hybridization and immunohistochemistry in a rat model, Stopera and coworkers found increased expression of mRNA and proteins of the oncogenes c-*fos and* c-*ras* in ACF (Stopera, Davie & Bird 1992a, Stopera & Bird 1992). c-*fos* mRNA is mostly located in the lower, proliferative compartment of crypts. The c-fos protein controls cell proliferation forming complexes with the c-jun protein (Halazonetis *et al.* 1988). The level of c-fos expression can be upregulated by all-*trans*-retinoic acid (Stopera & Bird 1993a). Moreover K-*ras* codon 12 point mutations were found in 7–37% of ACF examined (Stopera, Murphy & Bird 1992b, Vivona *et al.* 1993, Shivapurkar *et al.* 1994, Tochino *et al.* 1995), although Davies & Rumsby (1998) found no K-*ras* mutation in ACF from rats treated with AOM. Point mutations lead to inactivation of the K-*ras* oncogene, an event that is common in colorectal tumorigenesis (for review see Bos 1989). Interestingly, both ACF and K-*ras* mutations in transgenic mice can be prevented by enhanced expression of the MGMT gene, which encodes the O^6-alkylguanine-DNA alkyltransferase protein, a key enzyme in the mechanism of protection of DNA from methylating agents (Zaidi *et al.* 1995). However, it is not known whether or not K-*ras* mutations are pathogenetic for ACF in rodents.

Mutant p53 protein expression was immunohistochemically detected in rodent ACF (Stopera & Bird 1993b), though the specificity of monoclonal antibodies for the mutant p53 gene product is questionable. Twenty-seven of 65 ACF (42%) expressed a 'mutated' p53

protein, mainly in the cytoplasm of epithelial cells of aberrant crypts. The wild-type p53 protein inhibits cell proliferation, and it is also involved in the control of cell death. Thus it is possible that mutated p53 protein contributes to the induction of transforming phenotype in experimental ACF (expecially when dysplasia is evident). However, at present mutations in the *p53* gene have not been observed in experimental ACF (Davies & Rumsby 1998). Moreover, mutations in the *p53* tumour suppressor gene are events which occur relatively late in colon carcinogenesis (Vogelstein *et al.* 1988).

No mutation in the exon 15 of the *APC* gene in ACF of F344 rats was found using an *in vitro* synthezised protein assay (IVSP) (De Filippo *et al.* 1998). In this study however, no data on the histological features of the ACF examined were reported. The same result was obtained by Davies & Rumsby (1998) in Sprague–Dawley rats, although they found correlation of ACF multiplicity with carcinoma. Interestingly, however, MIN mice, heterozygous for a germline mutation of *APC*, when crossed with mice deficient of the *MSH*2 mismatch repair gene, developed many ACF, more rapidly growing adenomas, and had reduced survival, suggesting that somatic inactivation of the wild-type APC allele results in a faster progression of colonic carcinogenesis (Reitmar *et al.* 1996). Constitutional mutations in mismatch repair genes (Leach *et al.* 1993, Nicolaides *et al.* 1994, Papadopoulos *et al.* 1994) cause the Lynch syndrome (hereditary non-polyposis colorectal carcinoma (HNPCC)), which is associated with DNA instability at short (mono or polinucleotide) repeated sequences called microsatellites (Aaltonen *et al.* 1993, Ionov *et al.* 1993), which can be identified in tumours from affected individuals (for review see Marra & Boland 1995). Microsatellite instability (MSI) has been observed also in 10–15% of sporadic colorectal carcinomas (Aaltonen *et al.* 1993).

Overexpression of CD44, a class of cell surface glycoproteins, was found in dysplastic ACF of APC mutant mice and of patients with FAP, suggesting that CD44 is involved in the control of colonic epithelial cell dynamics (Wielenga *et al.* 1999). Finally, Kurimasa *et al.* (1999) have recently shown that DNA-dependent protein kinase (DNA-PK) may have a role in colorectal carcinogenesis. In fact null mice for the gene that encodes the catalytic subunit of DNA-PK develop ACF in their colon.

In conclusion, there is still uncertainty on the role played by oncogenes and tumour-suppressor genes in the induction and growth of experimental ACF.

HUMAN ABERRANT CRYPT FOCI

ACF in humans were firstly observed on the flat colonic mucosa of patients operated on for FAP, cancer or benign diseases of the large bowel (Pretlow *et al.* 1991, Roncucci *et al.* 1991a). Later, ACF were observed on the unsectioned mucosa using a dissecting microscope (Roncucci, Medline & Bruce 1991b). Topologically, the lumens of aberrant crypts showed various shapes which could be grouped into three categories (round, serrated, elongated), each predicting histological alterations (Roncucci *et al.* 1991b). The same features could be observed *in vivo* using a magnifying colonoscope (Takayama *et al.* 1998). The number of ACF per square cm of colonic mucosal surface is higher in patients with FAP. In these patients the ACF examined showed definite dysplasia at histology in 75–100% of cases (Roncucci *et al.* 1991a, Nucci *et al.* 1997), and could be appropriately referred to as microadenomas. They stain with *Dolichus biflorus* agglutinin and express the sialyl Lewis-a antigen at higher frequency than ACF from patients with cancer or benign diseases of the large bowel (Nucci *et al.* 1997). Microadenomas were identified also in random biopsies taken

during retrograde endoscopy from the distal ileum of patients with FAP who underwent total colectomy (Bertoni *et al.* 1995), although in this case the lesions were not evident during the endoscopic examination of the mucosa. On the other hand, ACF were identified *in vivo*, but using a mignifying colonoscope and after staining with methylene-blue, during endoscopy in patients with colonic tumours or with normal colon (Dolara *et al.* 1997, Takayama *et al.* 1998). The density of ACF is lower in patients with cancer and benign diseases of the large bowel (i.e. diverticular disease) as compared with patients with FAP. The density of ACF in patients with colorectal cancer is higher in the left colon and rectum than in the right colon (Roncucci *et al.* 1991b, Yamashita *et al.* 1995, Siu *et al.* 1997, Roncucci *et al.* 1998, Shpitz *et al.* 1998), although in the right colon they tend to be larger (Roncucci *et al.* 1998). ACF density does not seem to be related to sex. Older individuals harbour a slightly higher number of ACF in their colon. Moreover, patients with colon cancer resident in regions with high incidence rates of colorectal cancer have higher density of ACF in the colonic mucosa than patients from low incidence regions (Roncucci *et al.* 1998). All these data are in agreement with the anatomical distribution and some epidemiological characteristics of colorectal cancer in humans.

Histologically ACF are rather heterogeneous in patients with cancer or benign diseases of the colon. They may be normal (i.e. no cell or tissue abnormalities are evident) or show various alterations, from hyperplasia to severe dysplasia. Only a minor fraction of the ACF examined were defined as dysplastic, although in the literature a wide range of figures have been reported (5–54%) (Roncucci *et al.* 1991a, Jen *et al.* 1994, Otori *et al.* 1995, Yamashita *et al.* 1995, Di Gregorio *et al.* 1997, Nucci *et al.* 1997, Siu *et al.* 1997, Otori *et al.* 1998, Roncucci *et al.* 1998, Shpitz *et al.* 1998, Takayama *et al.* 1998, Bouzourene *et al.* 1999). The reasons of these differences are the following: the definition of dysplasia in colorectal pathology is not easy (Riddell *et al.* 1983); dysplasia may be focal in an aberrant crypt focus (Di Gregorio *et al.* 1997), and even in a single aberrant crypt (Siu *et al.* 1997), suggesting that a transition from hyperplasia to dysplasia in aberrant crypts is possible, maybe through cell proliferative alterations (Otori *et al.* 1995, Otori *et al.* 1998). It is noteworthy that an ACF with carcinoma *in situ* was also reported in a patient with colon carcinoma (Konstantakos *et al.* 1996). Dysplasia seems more frequent in larger ACF (Yamashita *et al.* 1995, Siu *et al.* 1997), although others found the opposite (Roncucci *et al.* 1998). The density of dysplastic foci was higher in the colon, expecially the right colon, whereas hyperplastic foci were more frequent in the rectum (Roncucci *et al.* 1998).

Scanning electron microscopy allowed a better definition of the surface features of human ACF (Vaccina *et al.* 1998). In particular the luminal openings of aberrant crypts on the mucosal surface are larger than normal (Figures 2a,b). The mucosal surface among aberrant crypts was flattened with loss of microvilli (Figures 3a,b), evident also with transmission electron microscopy (Figures 3c,d), indicating alterations at the cell surface of aberrant crypts.

At variance with rodents, human ACF have normal levels of hexosaminidase activity or even decreased levels (Pretlow *et al.* 1991). On the other hand, the expression of carcinoembryonic antigen (CEA) is enhanced (Pretlow *et al.* 1994b).

Human ACF have a hyperproliferative epithelium (Roncucci *et al.* 1993). Patients prior to an operation for colorectal cancer were given BrdUrd intravenously. The ACF harvested and examined after operation showed an increase of the total LI, though the proliferative compartment of aberrant crypts remained confined to the lower two thirds of the crypt. It is of note however, that all ACF examined were hyperplastic and not dysplastic on histological examination. Increased proliferative activity was found also by Otori *et al.* (1995). In that

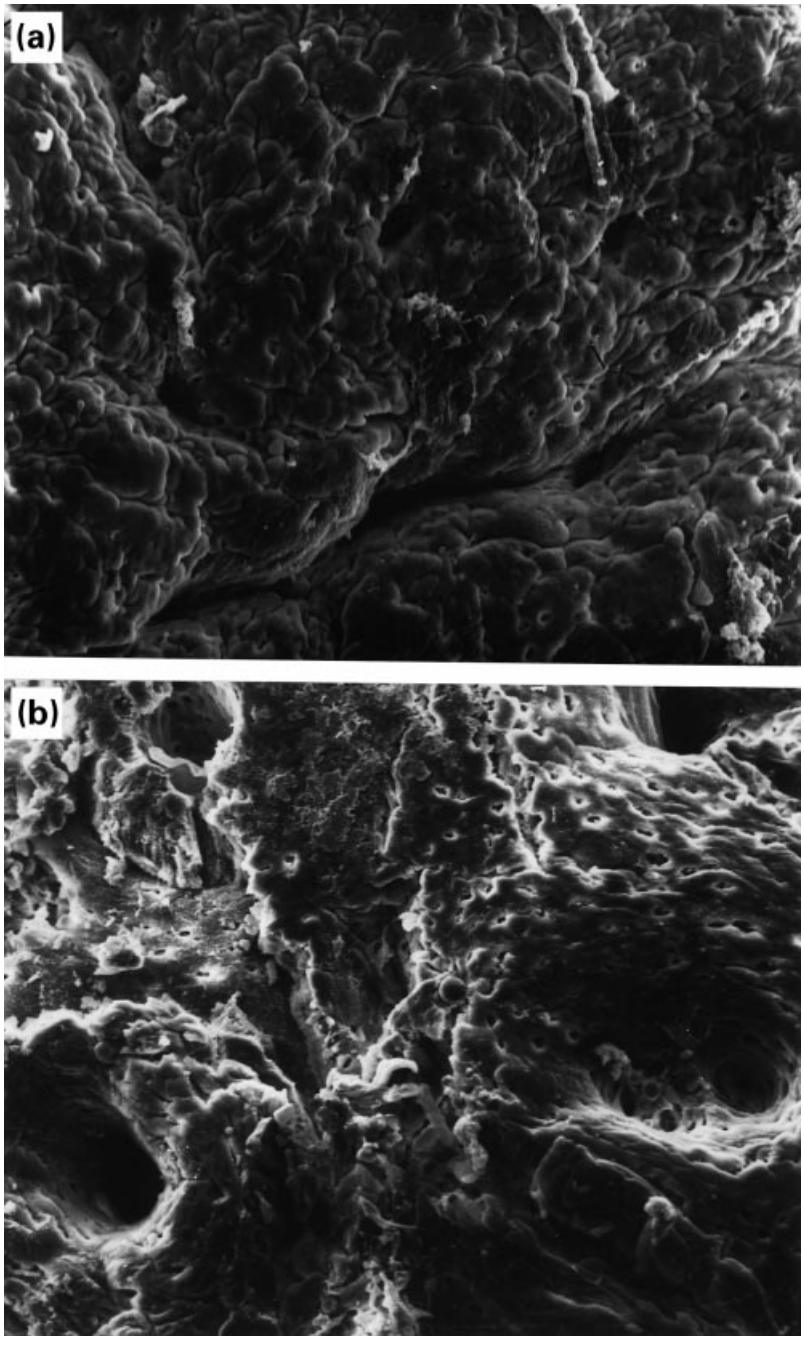

Figure 2. Scanning electron micrographs of the surface of normal colonic mucosa (a), and of aberrant crypts (b) at the same magnification (× 410). Arrows: luminal openings of crypts.

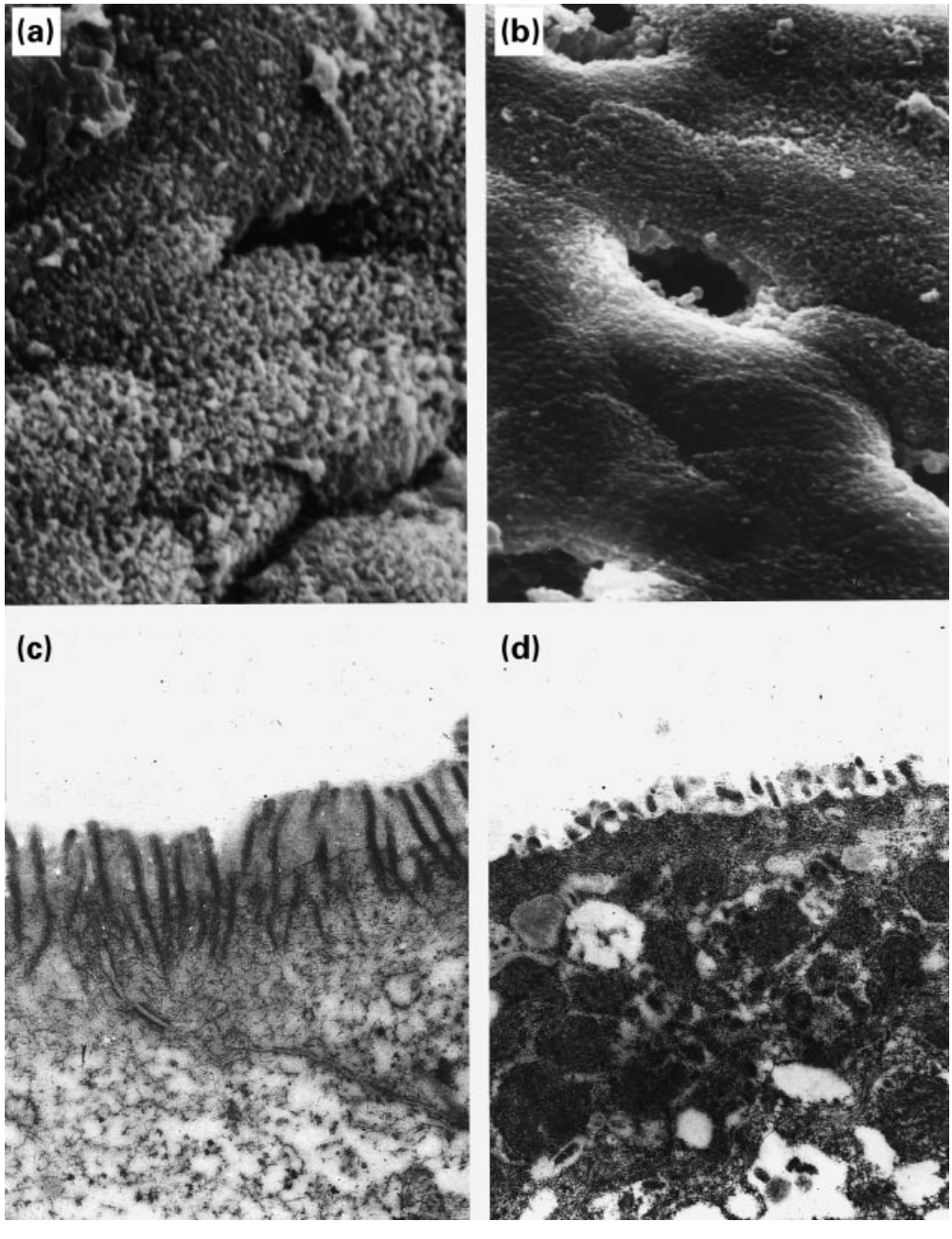

Figure 3. Scanning electron micrographs (a,b: magnification $\times 4000$), and transmission electron micrographs (c,d: magnification $\times 24\,000$) of human colonic mucosal surface. (a,c) Normal mucosa covered by numerous, well developed microvilli. (b,d) The surface of aberrant crypts is smoother owing to the presence of sparce and short microvilli.

work, a group of large ACF, called 'stage I abnormality crypts' which showed extension of the proliferative zone to the whole crypt, evaluated with immunohistochemical PCNA labelling, and were considered a transition from hyperplastic to dysplastic crypts. Also Shpitz *et al.* (1997), using the same method, found expansion of the proliferative compartment to the upper portion of aberrant crypts. This was true for hyperplastic and dysplastic crypts, though more evident for the latter.

The first genetic alterations reported in human ACF were mutations in K-*ras* (Pretlow *et al.* 1993, Smith *et al.* 1994, Losi *et al.* 1996). These mutations seem limited to hyperplastic ACF (Jen *et al.* 1994, Yamashita *et al.* 1995, Nucci *et al.* 1997), although Takayama *et al.* (1998) found K-*ras* mutations in more than 50% of dysplastic ACF examined. A weak correlation between ACF and carcinoma in the same patient was found for the presence, and for the type, of K-*ras* mutations (Losi *et al.* 1996) whereas others reported a stronger correlation (Shivapurkar *et al.* 1997). On the other hand, *APC* mutations are present only in dysplastic ACF, both in patients with FAP and colon cancer (Jen *et al.* 1994, Smith *et al.* 1994, Otori *et al.* 1998), although the frequency is low, suggesting that other genetic events may be involved in the first steps of tumour development.

Interestingly, dysplastic crypts in patients with FAP (with *APC* mutations) divide faster than normal crypts (Bjerknes *et al.* 1997). p53 protein accumulation and *p53* gene mutations were not found in ACF (Yamashita *et al.* 1995, Losi *et al.* 1996), with only one exception (Shivapurkar *et al.* 1997). However, a decreased and altered expression of $p21^{WAF1/CIP1}$ (whose transcription is regulated by p53) has been reported in dysplastic ACF from FAP patients, confirming an altered regulation of cell cycle control in human ACF (Polyak *et al.* 1996).

Moreover, microsatellite instability has been shown in a low fraction of human 'sporadic' ACF (Augenlicht *et al.* 1996, Heinen *et al.* 1996), suggesting that MSI can be an early event in tumour development. Finally, ACF, like many human tumours and at variance with normal mucosa, were shown to be monoclonal, by determining the pattern of chromosome X inactivation (Siu *et al.* 1999).

As mentioned above, a very important step toward the use of ACF as an early biomarker of susceptibility to colon cancer in humans has been the *in vivo* identification of foci during endoscopy, using a magnifying colonoscope (Dolara *et al.* 1997, Yokota *et al.* 1997). Takayama *et al.* (1998) demonstrated that it is also possible to test putative chemopreventive agents of colon cancer with ACF as early markers of increased risk.

CONCLUSIONS

The large body of work of the past decade has provided evidence that ACF are precursor lesions of both experimental and human colon cancer. Only a minor fraction of ACF will become cancer. It seems that larger ACF, with altered morphology (slit-like or elongated lumens at topology), dysplastic at histology, with altered cell kinetics, and with mutations in some genes involved in colorectal carcinogenesis are the most probable candidates for progression to carcinoma. Despite this, from a practical point of view, it is evident that ACF have most of the requisites needed by early biomarkers of cancer risk to be used in experimental and human intervention studies aimed at identifying agents able to reduce the incidence of colorectal carcinoma. Thus it is useful to look for ACF in rodent and human colons, in order to shed some light on the earliest events of colon carcinogenesis and to test measures to prevent colorectal cancer.

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